DATA EVALUATION RECORD

RPA 412636 (FENAMIDONE)

Study Type: §84-2; Mouse Lymphoma Cell/Mammalian Activation Gene Forward Mutation Assay (L5178Y *hgprt*)

Work Assignment No. 4-01-155V MRID 45386116

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells in Culture Gene Mutation Assay in L5178Y Mouse Lymphoma Cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: 046679 DP BARCODE: D278089
SUBMISSION NO.: S603761

TEST MATERIAL (PURITY): RPA 412636 (S-enantiomer of RPA 717879; 99.8% a.i.)

SYNONYMS: (S)-5-Methyl-5-phenylimidazolidine-2,4-dione

CITATION: Fellows, M. (1999) RPA 412636 (S-enantiomer of RPA 717879): Mutation at the *hprt* Locus of L5178Y Mouse Lymphoma Cells Using the Microtitre® Fluctuation Technique. Covance Laboratories Limited, North Yorkshire, England. Laboratory Report No.: 198/148-D5140, May 24, 1999. MRID 45386116. Unpublished.

SPONSOR: Aventis CropScience, 2 T.W. Alexander Dr., Research Triangle Park, NC

EXECUTIVE SUMMARY: In two independent trials of a mammalian cell gene mutation assay at the L5178Y *hgprt* locus (MRID 45386116), mouse lymphoma cells cultured *in vitro* were exposed to RPA 412636 (S-enantiomer of RPA 717879, 99.8% a.i.; Lot/Batch #: LPO-348), in dimethylsulfoxide (DMSO) for 3 hours at concentrations of 100, 200, 400, 800, or 1600 µg/mL (Trial 1) and 400, 800, 1200, or 1600 µg/mL (Trial 2), both in the presence and absence of mammalian metabolic activation (+/-S9).

RPA 412636 was tested up to the limit of solubility (1600 µg/mL +/-S9). Slight to moderate cytotoxicity was observed at 1600 µg/mL in the presence and absence of S9, in both trials. No statistically significant increases in mutant frequency compared to solvent controls were observed at any dose level in the presence or absence of S9-activation in either trial. No linear trend in mutation frequency with dose was detected. The positive controls induced the appropriate responses. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: RPA 412636 (S-enantiomer of RPA 717879)

Description: White powder
Lot/Batch #: LPO-348
Purity (w/w): 99.8% a.i.
CAS # of TGAI: Not provided
Structure: Not provided
Solvent used: Dimethylsulfoxide (DMSO)

2. Control materials

Negative control: The solvent alone served as the negative control.
Solvent control: DMSO (0.2 µg/mL diluted 100 fold in culture medium)
Positive controls
Non-activation: 4-Nitroquinoline-1-oxide (0.1 and 0.15 µg/mL in DMSO)
Activation: Benzo(a)pyrene (2 and 3 µg/mL in DMSO)

3. Activation: S9 derived from male Sprague-Dawley rats.

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			None		Hamster		Other (name)
			Other (name)		Other (name)		

The S9 fraction was purchased from Molecular Toxicology Inc., USA and was stored at -80°C prior to use. The S9 fraction was checked by the manufacturer for sterility, protein content, cytochrome P-450-catalyzed enzyme activities, and the ability to convert ethidium bromide, cyclophosphamide, 2-aminoanthracene, and benzo(a)pyrene to bacterial mutagens. The S9 mix was prepared by mixing glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), KCl (150 mM), and S9 fraction in the ratio of 1:1:1:2. The final S9 culture concentration was 2%.

4. Test cells: Mammalian cells in culture

X	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		others (list)

Media: Three types of RPMI 1640 medium: RPMI 10 (growth medium), containing 10% (v/v) heat inactivated horse serum, gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL), and pluronic (0.5 µg/mL); RPMI A (treatment medium), supplemented with gentamycin (100 µg/mL), amphotericin B (2.5 µg/mL), and pluronic (0.5 µg/mL); and RPMI 20 (plating medium), containing 20% (v/v) heat inactivated horse serum, gentamycin (100 µg/mL), and amphotericin B (2.5 µg/mL).

Properly maintained?	X	Yes		No
Periodically checked for mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5. Locus examined

		Thymidine kinase (TK)	X	Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na^+/K^+ ATPase
Selection agent:		bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG) (15 µg/mL)		
		trifluorothymidine (TFT)				

6. Test compound concentrations used

a. Preliminary cytotoxicity assay

Non-activated conditions: 0, 25, 50, 100, 200, 400, or 800 µg/mL
Activated conditions: 0, 25, 50, 100, 200, 400, or 800 µg/mL

b. Mutagenicity assays

Non-activated conditions: 0, 100, 200, 400, 800, or 1600 µg/mL (Trial 1); 0, 400, 800, 1200, or 1600 µg/mL (Trial 2)
Activated conditions: 0, 100, 200, 400, 800, or 1600 µg/mL (Trial 1); 0, 400, 800, 1200, or 1600 µg/mL (Trial 2)

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 3 hours (non-activated) and 3 hours (activated).

b. After washing, cells were cultured for 7 days (expression period) before cell selection.

c. After expression, $2x10^4$ cells/well (384 wells/group) were cultured for 12 days in selection medium to determine numbers of mutants and 1.6 cells/well (192 wells/group) were cultured for 8 days without selective agent to determine cloning efficiency.

2. Statistical methods: The following statistical methods were applied to the data:

Parameters Investigated	Statistical Test
Mutation frequency	Log transformation of data followed by Dunnett's test ($p \leq 0.05$) and Chi-square (one-tailed linear trend analysis; $p \leq 0.05$, or 0.01). Procedures followed UKEMS guidelines (Robinson *et al*, 1990).

The reviewer considers the statistical analyses used to be appropriate.

3. Evaluation criteria

a. Assay validity: The assay was considered valid if all of the following criteria were met:

- The mutant frequencies of the solvent controls were within the normal range.
- At least one concentration of each of the positive controls induced a clear increase in mutant frequency.

b. Positive result: The test article was considered mutagenic if all of the following criteria were met:

- The assay was valid.
- The mutant frequency at one or more doses was significantly greater than solvent controls ($p \leq 0.05$).
- There was a significant dose-response as indicated by the linear trend analysis ($p \leq 0.05$).
- The effects were reproducible.

II. REPORTED RESULTS

Dose formulations were not analyzed for actual concentrations.

A. PRELIMINARY CYTOTOXICITY ASSAY: In the range-finding assay, mouse lymphoma L5178Y cells (1 culture/dose) were exposed to the test material for 3 hours at concentrations of 25, 50, 100, 200, 400, or 800 µg/mL (±S9). Neither marked toxicity nor precipitation was observed at any dose level in the presence or absence of S9-activation. Based on these results, five doses ranging from 100-1600 µg/mL were chosen for the first mutagenicity assay.

B. MUTAGENICITY ASSAY: The results of the mutagenicity assays were summarized in the Study Report, Table 2, page 24 and are included as an ATTACHMENT to this DER.

In both trials, a precipitate was observed at 1600 µg/mL in the presence and absence of S9 after the incubation period. In the first mutagenicity assay, duplicate cultures of mouse lymphoma L5178Y cells were exposed to the test material for 3 hours at concentrations of 100, 200, 400, 800, or 1600 µg/mL (±S9). Slight toxicity was observed at the high dose (1600 µg/mL) as indicated by the relative survival values of 69% (-S9) and 73% (+S9). No statistically significant increases in mutation frequency compared to solvent controls were observed at any dose level in the presence or absence of S9. No linear trend in mutation frequency with dose was detected. In trial 2, concentrations of 400, 800, 1200, or 1600 µg/mL (±S9) were used. Slight to moderate cytotoxicity was observed at the high dose (1600 µg/mL) as indicated by the relative survival values of 63% (-S9) and 58% (+S9). No statistically significant increases in mutation frequency compared to solvent controls were observed at any dose level in the presence or absence of S9. No linear trend in mutation frequency with dose was detected. The positive controls (4-nitroquinoline-1-oxide, -S9 and benzo(a)pyrene, +S9) induced significant increases in mutant frequency at all doses in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The investigator concluded that RPA 412636 did not induce mutation at the *hprt* locus of L5178Y mouse lymphoma cells when tested up to the limit of solubility (1600 µg/mL), in the presence or absence of S9-activation.

B. REVIEWER COMMENTS: RPA 412636 was tested up to the limit of solubility (1600 µg/mL ±S9) in two trials. Slight to moderate cytotoxicity was observed at 1600 µg/mL in the presence and absence of S9, in both trials. No statistically significant increases in mutant frequency compared to solvent controls were observed at any dose level in the presence or absence of S9-activation in either trial. The positive controls induced significant increases in mutant frequency compared to solvent controls. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

C. STUDY DEFICIENCIES: The following minor deficiencies were noted, but would not change the conclusions of the study:

- Dose formulations were not analyzed for actual concentrations.
- Historical control data were not provided.